METHODS OF FORAGE EVALUATION

by

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DEDICATION

To my mother, Alice Namuddu Bintubizibu, who sacrificed so much for the sake of my education.

TABLE OF CONTENTS

		Page
1.	Introduction	1
2.	Indicator Techniques in Digestibility Studies	2
3.	Use of Intact Animal (in vivo) in Forage Evaluation	14
4.	Digestion Techniques Using Rumen Microflora in vitro	28
5.	Prediction of Forage Value from Chemical Analyses	43
6.	Forage Evaluation from:	
	a) Comparative Feeding Trials	61
	b) Rumen Concentration of Volatile Fatty Acids	65
7.	Summary	69
8.	Acknowledgments	73
9.	Literature Cited	74

INTRODUCTION

Farm animals, particularly the ruminants, have the ability to convert forages not generally consumed by humans into food and wool for human use. Forages constitute an important and big part of the ration of ruminant animals even in countries like the United States of America where considerable amounts of concentrates are fed. In many other parts of the world, particularly the developing countries, the entire ration of farm ruminants may consist of forages especially in the form of pasture. The forages usually fed include hay, silage and pasture.

As forages are such an important feed in livestock production, various attempts have been made to devise methods of assessing their nutritive value. The methods of forage quality evaluation have ranged from visual observation and chemical analyses to in vivo techniques. The development of reliable methods of estimating forage quality is of importance not only to the livestock producer but also the plant breeder, the agronomist and the animal nutritionist.

The aim of this report was to review and summarize the literature on experiments which have been carried out by various investigators with regard to methods of forage evaluation. In this report no attempt will be made to review work on the methods of quantitative forage evaluation, e.g., forage yield per square unit of land.

INDICATOR TECHNIQUES IN DIGESTIBILITY STUDIES

In the conventional digestibility studies the amounts of food eaten and the feces excreted are actually weighed. This, however, is a laborious and time-consuming method and the number of trials which can be run is greatly limited. Thus indicators have been used to estimate the above two quantities.

Assuming the indicator is indigestible, then the amount excreted must equal the amount ingested. Using KLEIBER's notation (1961)

$$I.\quad C_i = F.C_f$$

$$\frac{F}{I} = \frac{C_i}{C_f}$$

where C_i = the concentration of indicator in the food

I = daily food intake

F = amount of feces excreted per day

C_f = concentration of the tracer in feces.

The digestibility D is defined by

$$\frac{I-F}{I}$$

$$D = 1 - \frac{F}{I}$$

Substituting $\frac{C_i}{C_f}$ for $\frac{F}{I}$

$$D = 1 - \frac{C_i}{C_f}$$

Dry matter consumed

$$= \frac{\text{Weight of feces x \% indicator in feces}}{\text{\% indicator in forage}}$$

Fecal dry matter output

$$= \frac{\text{Amount of indicator fed}}{\text{\% indicator in feces sample}}$$

A naturally occurring indicator in the feed such as lignin or nitrogen may be used. Using KLEIBER's notation (1961) if F and I are same as above

and n_i = concentration of internal indicator in feed

n_f = concentration of internal indicator in feces

then apparent digestion coefficient is

$$\frac{I.ni - Fn_f}{Ini}$$

$$= \frac{1 - Fn_f}{In_i}$$

Substituting $\frac{C_i}{C_f}$ for $\frac{F}{I}$

$$\text{Apparent digestion coefficient} = 1 - \frac{C_i \cdot n_f}{C_f \cdot n_i}$$

$$= 100 - \frac{100 \times \text{\% indicator in feed} \times \text{\% nutrient in feces}}{\text{\% indicator in feces} \times \text{\% nutrient in feed}}$$

The various types of external indicators which have been used are ferric oxide, barium sulphate, monastral blue, titamium oxide, polyethlene glycol, radioactive tracer, a dye-anthroquinone, and chromic

oxide (Raymond and Minson, 1955). Raymond and Minson (1955) gave the requirements of a tracer as follows: it should be quantitatively recovered in the feces, non-toxic, inexpensive, readily analyzed by physical or chemical methods and present only in small amounts in the original diet. The literature indicates that chromium oxide has been most studied and its usefulness in grazing experiments has been demonstrated (Reid, 1962).

Stevenson (1962) observed that the accuracy of fecal output estimate depends on two factors; the quantity of chromium oxide excreted in the feces and this should be equal to the quantity fed over a given period and secondly, the sample of feces taken for marker determination should be representative of the total fecal output. Most studies, therefore, on the use of chromium oxide in digestibility trials have concentrated on the degree of recovery of the chromium oxide and excretion pattern of the indicator and method of sampling feces.

Raymond and Minson (1955) tried to overcome diurnal variations in chromium oxide concentration in the feces by feeding it in a dispersed form as the latter would mix better with feed than when fed in capsules. The results of the study did not give evidence of any decrease in the range of diurnal variation. These workers noted that small losses of chromium oxide occurred during drenching and dosing numbers of animals with drench was more difficult than with capsules. From their study Raymond and Minson (1955) concluded that there is no standard excretion pattern on which "grab" sampling of feces could be based. Stevenson (1962) also reported considerable week-to-week variation from both

representative and grab samples. This emphasized the need for long-term trials if errors caused by unknown factors are to be reduced to a minimum.

Raymond and Minson (1955) described the technique of "ring sampling" which they thought was a practicable method of obtaining a sample of feces from grazing stock which is representative of feces voided during 24 hours. When they compared fecal production estimated by total collection and by ring sampling for chromium oxide, an average difference (B - A) of 4.6% was found, where B = estimate from chromium oxide

A = actual production

Stevenson (1962) reported that in representative samples 97.4% of the chromium oxide was recovered, the low recovery being attributed to a loss of chromium oxide when the feces were ground. He suggested therefore that a correction for low recovery should be applied according to specific conditions operating when estimating fecal output of chromium oxide.

Lambourne and Reardon (1963) reported that the trend toward diurnal cycle in chromium oxide concentration was reduced when the marker was administered twice daily. They also observed that there is no reproducible pattern of chromium oxide excretion for all animals in a group from day-to-day even in the well-controlled environment of hand fed stock. They thus concluded that the fecal output of an individual sheep over the range of 50 - 150 grams of organic matter per day may be estimated within ± 12% over periods of 10 days or more by the orthodox chromium oxide capsule method with two doses and two samples per day.

They suggested that any convenient time for sampling should prove satisfactory.

Langlands et al. (1963a) found from their study that the variability in estimates of daily feces output tended to be less for heifers given paper than those given capsules. They concluded that feeding chromium oxide in paper is to be preferred to capsules when grab sampling is practiced, but the advantage of paper when sward sampling is practiced may be slight. In an earlier study Langlands et al. (1963b) had come to a similar conclusion that errors of estimate of fecal output were more stable when chromium oxide was administered in paper instead of in capsules.

Canadian researchers, Fisher et al. (1965) conducted experiments to substantiate and extend the information on the use of sustained release pellets with ruminants. Sustained release pellets were studied in an attempt to reduce variations in chromium oxide excretion. In this study in vitro experimental percentage weight loss (y) was regressed on initial weight (x), $y = 2.97 x - 23.92$, ($r = .73$ S.D. = 2.73). These results indicated that success in maintaining a constant level of chromium oxide could be expected were the pellets to perform in a similar manner in the reticulum. In this study the regurgitation percentage of the pellets was 4.5 Fisher et al. (1965) noted that sustained release pellets were effective in reducing diurnal variation, but they expressed some doubt as to whether there was uniform mixing of chromium oxide in the rumen contents. The chromium oxide possibly remained as a suspension in the fluid portions of the ingesta rather than adhering to the particles of the ingesta.

The percentage difference between the indicator method estimate and the total collection method was 12.7 for estimating dry matter digestibility and 9.7 for energy digestibility (Fisher et al., 1965). The percentage difference between calculated and actual dry matter intake was 35.3. Their overall conclusion was that sustained release pellets as prepared in their study were not satisfactory for administering the chromium oxide indicator to estimate digestibility or feed intake.

Christian et al. (1965) conducted experiments to obtain further information on the reliability of the marker technique under stall feeding conditions and appraise major sources of error in estimation of feces output. The results of their study showed that over the whole period marker recovery was only 92.8 percent. Statistical analysis indicated that marker recovery fluctuated significantly as between periods but not as between sheep. The overall difference (bias) between marker recovered and marker fed was highly significant. Christian et al. (1965) also observed that fluctuations in marker recovery did not appear to be directly related to changes in either feed intake or feces output.

Lambourne and Reardon (1963) had also reported that calculations of various correlations failed to show any systematic relationship between daily organic matter intake or output and chromium oxide on the same or following day. This suggested that indicator concentrations did not reflect minor day-to-day variations in intake or output (Lambourne and Reardon, 1963).

Christian et al. (1965) studied feces organic matter output estimated from marker concentrations in daily representative samples, "grab" samples bulked on equal dry matter basis for each sheep over three days and "grab" samples bulked for each half-day over all sheep. In each case the estimates significantly exceeded the actual feces by approximately 10% reflecting the incomplete marker recovery.

Six yearling Angus steers in a single reversal digestion trial were used to study effects of frequency of feeding on diurnal excretion of chromium oxide, crude protein, and gross energy (McGuire et al., 1966). The results of this study showed that digestion coefficient calculated from chromium oxide were lower than those calculated by the conventional method because only 94.2% of the chromium oxide was recovered. A significant (P = .01) difference in chromium oxide concentration was noted among steers.

Since the indicator method of determining digestibility is based on the ratio of the nutrient in question and indicator at the time of sampling, excretion rates of both nutrient and indicator may affect digestibility calculations (McGuire et al., 1966). The results of McGuire et al's (1966) study showed that excretion rates chromium oxide and crude protein were significantly (P .01) correlated. Gross energy curves suggested that gross energy excretion fluctuates very little throughout the day and that reliable digestion coefficients can be determined by sampling at any time of the day.

The relationship between fecal excretion of nitrogen and dry matter intake has been used in grazing experiments. Reid (1962) reviewed work which had been done on use of nitrogen and plant chromogens

in the ratio and fecal index technique in digestibility studies. He concluded that from the point of view of its application in grazing experiments, nitrogen was useful only as a fecal indicator.

Marten et al. (1963) carried out experiments to test the applicability under Minnesota conditions of two fecal index equations for estimating pasture digestibility. The equations were:

(1) $Y = (0.0925X + 137.34 \text{ Log } X) - 242.12$

where Y = forage chromogen

X = feces chromogen

(2) $Y = 0.97X + 1.02$

where Y = feed to feces ratio

X = nitrogen content of feces

Digestibility estimates by the chromogen technique compared to the nitrogen technique resulted in correlation coefficients of 0.48 and 0.41 in 1958 and 1959, respectively. These coefficients were highly significant ($P = .01$) although they accounted for only 23% and 17% of the variation. Marten et al. (1963) further noted that the two techniques were equally sensitive statistically in detecting any differences caused by pasture forage mixtures.

Lambourne and Reardon (1963) observed that fecal nitrogen concentrations were less variable than chromium oxide concentration, but changes followed the same pattern as shown by chromium oxide concentration; lagging by several days behind the change in feeding regime in some cases.

Anthony et al. (1954) reported that for forages they studied the fecal chromogen technique was a reliable method for estimating forage

digestibility. Reid (1962) reviewing the literature on lignin, methoxyl and "macerate" crude fiber came to the conclusion that there was very little merit in attempting to replace nitrogen or plant chromogen as internal indicators with any substance, such as lignin, methoxyl or crude fiber whose concentration was inversely related to digestibility. Van Dyne and Meyer (1964a) reported that estimates of forage intake by lignin ratio technique were significantly lower than those obtained by using in vivo cellulose microdigestion technique.

Hutton and Jury (1964) examined the effect of feed availability on the feed/feces-fecal nitrogen relations. They related feed/feces ratios by regression analysis. Prediction errors were higher under full than under restricted feeding although similar regression coefficients applied to each level. It was also reported that errors were lowest at both feeding levels when fecal energy content rather than nitrogen was used to estimate feed/feces ratio. The chemical determination of nitrogen is more readily undertaken in the laboratory. In some circumstances, therefore, the ability to make more observations when nitrogen is used compensate for the slightly higher error associated with its use in regression equations (Hutton and Jury, 1964).

Lambourne and Reardon (1963a) investigated the probable magnitude of errors involved in the use of chromium oxide--nitrogen technique. They reported that chromium oxide-nitrogen method tended to underestimate the higher intakes slightly, but attributed this to an aberation of sampling from a population of digestion trials they carried out. Prediction intake factor from grab samples may be expected to contribute about three-quarters of the error variance in estimates of feed intake

and remains a potential source of bias (Lambourne and Reardon, 1963a).

Owen (1961) found that a "dissolved feces fraction" (D.F.F.), a fraction extracted from the feces on standing it in 0.2N HCl for 18 hours, was related to dry matter intake by the equation $y_1 = 250 + 17.8\ x_1$ where y_1 = daily dry matter intake (gram)

x_1 = quantity of D.F.F. excreted (gram)

Langlands and Corbett (1964) used feces samples obtained from a number of continuous digestibility trials to evaluate Owen's method.

From their study Langlands and Corbett (1964) obtained the following equation $y_1 = 465 + 17.8\ x_1$ (y_1 and x_1, same as above). The workers criticized this linear relationship between dry matter intake and total excretion of D.F.F. because it implied no D.F.F. at intakes less than 465 g dry matter per day. They, therefore, suggested that the logarithmic form of the relationship,

$$y_2 = 1.6734 + 0.907\ x_2 \pm 0.2060\ S.E._b = 0.31.$$

where $y_2 = \log_{10}$ daily dry matter intake (gram)

$x_2 = \log_{10}$ daily total excretion of D.F.F. (gram) was more acceptable on both physiological and statistical grounds. Langlands and Corbett (1964) showed that in general the precision of the D.F.F. method was not as high as that of the fecal nitrogen for individual growth herbage. Their overall findings, did not endorse the findings of Owen (1961) that the D.F.F. would provide a fecal index applicable to a wide range of herbage and animals and would overcome the need to derive specific equations for estimating intake restricted to individual growth of herbage.

Raymond (1966) remarked that the conceptual validity of the fecal index method was itself in question. The basic assumption that a mathematical relationship exists between the digestibility data of cut samples of whole herbage and the digestibility data for the fraction of the sward which the animal grazes in the field, appears to be invalid at least in the cases of fecal nitrogen and chromogen (Raymond, 1955). Lambourne and Reardon (1962) showed that the "stem" and "leaf" fractions of the same sward gave rise to quite different digestibility/fecal nitrogen relationships. Alder and Minson (1963) derived the following equation relating herbage organic matter digestibility to fecal nitrogen concentration.

Digestibility of Herbage Organic Matter

= 51.15 + 6.84 (% nitrogen in fecal organic matter)

S.E. ± 2.86

Digestibilities estimated from the above equation were compared with the measured digestibilities. Analysis of variance showed that nearly 90% of the deviations may be accounted for in terms of feed variation. Alder and Minson (1963) pointed out that errors arise when data from indoor digestion trialsare applied to animals in the field. Observations of forage consumed suggested that the cattle selected the ends of leaves and tops of stems including flower heads. The digestibility of these fractions could have been higher than the estimates obtained (Alder and Minson, 1963).

From the literature reviewed it appears that chromic oxide is the external indicator used in almost all the digestibility studies reported

here. The main limitations on its use are low degree of recovery and an unpredictably variable pattern of excretion in the feces. More studies are needed to establish a more reliable method of sampling feces. Nitrogen and plant chromogens are the most favored internal indicators. Owing to the selective nature of the grazing animal, mathematical relationships of fecal nitrogen or plant chromogens to herbage digestibility derived from indoor trials should be applied with caution to grazing animals in the field.

USE OF INTACT ANIMAL (IN VIVO) IN FORAGE EVALUATION

The intact animal as used in conventional digestion trials has played an important part in forage evaluation studies. As far as forage studies are concerned, most conventional digestion trials have used sheep and cattle. Other species of animals, however, have been tried. Byer et al. (1964) conducted studies to compare the goat to the steer for use in measuring forage digestibility. Their studies showed that goats and steers exhibited very similar digestive abilities. The only difference was in protein digestibility. The overall conclusion from the study was that the goat could be used to evaluate the digestible nutrients or energy content of forages. Recently Adegbola et al. (1966) carried out experiments to provide further information on the dependability of rabbits as test animals in the evaluation of forage quality. From this study it was reported that the use of rabbits to obtain feed weight conversion data should be viewed with skepticism until more information has been developed.

Digestibility values of many forages have been calculated by averaging data obtained by use of sheep in some studies and cattle in others. Swift and Bratzler (1959) initiated research to answer the question whether digestibility values obtained with sheep were applicable to cattle. The average of all digestible dry matter, digestible protein, and digestible energy values as obtained with sheep were: 61.5 percent, 65.9 percent and 2656 calories per kilogram dry matter respectively. The corresponding average values as obtained with cattle were: 62.1%, 64.6% and 2623 calories per kilogram dry matter respectively.

Statistical study of data showed no significant difference at the 5% level in the digestive capabilities of these two species of animals. Donefer (1966) came to a similar conclusion that there was good agreement between the digestibility coefficients and TDN values obtained with sheep as compared to cattle.

Van Dyne and Wier (1964b) reported that on the range sheep had higher in vivo digestibility than cattle. This may result from sheep grazing higher quality forage than did cattle, thus sheep should have developed a denser and more vigorous microbial population (Van Dyne and Wier, 1964b). Howes et al. (1963) presented data obtained by using 12 non-pregnant purebred Hereford and Brahman heifers between ages of 2 and 3 years in a series of total collection digestion trials. They found that Brahmans had a higher coefficient of apparent digestion for all parameters except ether extract. Only coefficients for crude protein were significantly different (P .05). The factors which caused differences in digestion between different species of cattle were discussed (Howes et al., 1963). These studies indicate that feeding standards obtained with temperature breeds of cattle should be applied with caution to tropical breeds of cattle.

Harnesses and Collection Equipment

The conventional digestion trial requires total collection of feces (if indicator methods are not used) and, in some cases, urine. Harnesses and bags designed to make separate collection of feces and urine from grazing wethers have been described by Cooke et al. (1952) and Erwin et al. (1959). Gorski et al., (1957) and Lesperance and Bohman (1961) developed a portable apparatus that collects urine and

feces from cows and heifers. A collection apparatus developed to procure quantitative recoveries of urine from male cattle while they grazed pasture or range was described by Border et al. (1963). Wainman and Peterson (1963) described equipment for collection of urine from male cattle and sheep. Owen et al. (1962) described method and equipment for collecting from ewes.

Arnold (1960) reported his field experience with harnesses for total collection of feces from grazing ewes and wethers. Arnold (1960) commented on the need for a harness of reasonable cost and gave the requirements of such harness as follows. It should (i) enable complete collection of all feces voided; (ii) be simple and speedy to operate; (iii) cause minimum distress to the animal so that the sheep accustomed to wearing a harness and collection bag behaves normally. These requirements could also be extended to harnesses for cattle. Hughes (1963) drew attention to the problems associated with separating and collecting dung and urine from milking cows on metabolism trials. He also outlined the limitations of the apparatus that were then currently used for this purpose and described an equipment designed specifically for collecting excreta from Jersey cattle fed exclusively on fresh herbage. The specifity of the apparatus described limits the scope of its use in many digestibility studies.

Although the use of total fecal collection in digestibility trials eliminates errors associated with markers, Hutchinson (1956) suggested that the fitting of collection bags may interfere with normal grazing behavior and result in decreasing fecal outputs in consecutive days. Hadjipieris et al. (1965) reported that an analysis of variance of the daily fecal outputs in their study failed to show any such effect and

concluded that with the short collections they employed the fecal output was not seriously affected by the bags. Noblitt et al. (1963) attempted to assess the effect of employing the total collection technique (using attendants) upon nutrient digestibility by dairy cattle. The results showed that imposing the stress of manual total collection of feces on dairy cattle caused small but significant increases in the digestibility of dry matter and all of the components of dry matter. Noblitt et al. (1963) discussed the possible causes of increased digestibility in stressed animals but indicated that further studies were needed to more clearly define the effect of stress on nutrient utilization.

Use of Fistulated Animals

In forage quality studies the investigator is faced with the problem of obtaining a representative sample of the forage consumed by the animal. The sample may be analyzed for botanical composition, chemical composition, and/or digestibility using various parameters.

Investigations have shown that animals graze selectively (Hardison et al., 1954 and Meyer et al., 1957). Mowat et al. (1965) have demonstrated differences in the in vitro digestibility and protein content of leaf and stem portions of the species and varieties of the grasses and alfafa they studied. The digestibility of cut herbage does not necessarily correspond to the digestibility of grazed forage and in effect due to selective grazing changes in the digestibility of herbage consumed may be smaller than the changes for the growth of herbage as a whole (Corbett et al., 1963). The following methods have been used to obtain forage samples representative of ingested material under pasture

and range conditions. (1) hand-plucking, (2) harvesting before and after grazing, (3) sampling from fistulated animals. Cook (1964) presented a brief review on the use of the above methods.

Lesperance (1960) suggested that the only practical means of pasture and range forage evaluation was through the use of the animal as a biological sampling agent. Forage sampling using the animal was accomplished by Torrel (1954) with the establishment of a successful esophageal fistula in sheep. Van Dyne and Torrel (1963) reviewed the development and use of the esophageal fistula. Balch and Cowie (1962) described the procedure for establishing rumen fistulas in cattle and of care and maintenance of animals with fistulas. Bohman and Lesperance (1967) discussed the advantages and disadvantages of esophageal and rumen fistulas.

Wier and Torrel (1959) studied selective grazing by sheep by comparing the chemical composition of forage samples obtained by hand-clipping and by esophogeally fistulated sheep. The results showed that sheep consistently selected forage higher in protein and lower in crude fiber than that obtained by hand clipping. Correlation and regression studies indicated that it was not feasible to estimate what a sheep would eat from hand clipped materials (Wier and Torrel, 1959).

Edlefsen et al. (1960) reported that differences in chemical composition of diets obtained by fistula and hand-plucking were statistically significant for all components except for ether extract, total protein and cellulose. They also showed that animals grazing mixtures were, to a large degree, able to hold the nutritive levels of their diets constant during the trial period by shifting from species to species. Uniformity of results among fistulated sheep indicated that only a few

animals would be required for a satisfactory diet estimate. Langland's (1966) results confirmed that extrusa collected from esophageal fistula differed chemically from the herbage eaten.

The formation of a fistula in the esophagus or rumen of cattle makes possible the direct collection of freshly swallowed herbage, the digestibility of which can be determined by in vitro method. Tyler and Deriaz (1963) reported that the mean digestibility of herbage ingested was higher than that of the herbage offered by 14 units in one treatment and by up to 20 units in another. Tyler and Deriaz (1963) suggested that for certain studies of herbage intake this method would be preferable to the use of the fecal-index technique which is of doubtful validity to the study of within sward differences. An advantage of a rumen fistula rather than an esophageal one for studies of nutritive value within a sward is that the same experimental cattle can be used (although at different times) to obtain samples of rumen liquid for analysis of volatile fatty acids content. As it is shown later in this report volatile fatty acids analysis is receiving serious consideration as a parameter in forage evaluation.

Lesperance et al. (1962) found that the amounts of protein and ether extract were the same in the fistula sample as the feed, but significant changes were noted in the amount of crude fiber, nitrogen free extract and energy. The most striking changes in the chemical composition of fistula samples occurred in the mineral matter (Lesperance et al., 1962 and Langlands, 1966).

Langlands (1966) calculated regression equations to predict the digestibility and nitrogen content of the feed from the composition of

the fistula sample. The most precise relationship for estimating the digestibility of the feed was:

$$D_F = 9.60 + 0.840D_2 \pm 3.43$$
$$(\pm\ 0.049)$$

where D_F = predicted organic matter digestibility of the feed.

D_2 = estimate of the in vivo digestibility of the sample predicted from an in vitro digestibility but adjusted to include the organic matter in the liquid fraction which was regarded as being completely digestible.

Langlands (1967b) noted that the estimates of digestibility obtained by the fistula technique were in reasonable agreement with those calculated from the general relationship between the intake factor and nitrogen.

Langlands (1965) observed statistically significant diurnal trends in the nitrogen content of the diet selected by grazing sheep. He thus went on to comment that it would be prudent to consider the possibility of diurnal trends when designing experiments to examine selection by grazing sheep. Further work will be necessary to ascertain whether the changes observed reflect diurnal changes in the composition of the herbage organic matter or in the parts of the plant or species of plants selected (Langlands, 1965). Langlands (1967a) discussed the sampling problems introduced by diurnal changes in the composition of the material selected.

Lesperance et al. (1960) commented that since a variation exists between types of feed, with a high correlation between the composition of feed and fistula samples, the fistula samples probably represent the

best estimate of the composition of grazed forage until adequate comparisons of grazed forage or forage similar to grazed have been made. It is doubtful if regression equations calculated from hay and fistula samples could correctly apply to succulent feeds (Lesperance et al., 1960). Arnold et al. (1964) showed from their study that sheep with esophageal fistulas could have normal grazing times, estimated herbage intakes and productivity similar to normal sheep. Results from pen feeding studies indicated that the chemical composition of the esophageal samples collected, with the exception of ash, was similar to that of the diet (Arnold et al., 1964). Arnold et al. (1964) concluded that provided certain criteria are observed and animal variability allowed for, precise data on the diet of the grazing animal could be obtained from the use of sheep with esophageal fistulas. Robards and Wilson (1967) remarked that since sheep have been shown to be highly selective in their grazing there is little possibility of cutting accurate samples of what they eat from a mixed sward. The material separated out from fistula samples is the most accurate sample that can be obtained of the species eaten (Robards and Wilson, 1967).

Use of Frozen and Artificially Dried Pasture Herbage

Raymond et al. (1949) discussed the possible errors and difficulties found in conventional digestibility trial with cut herbage. One of the disadvantages they pointed out was that during the feeding trial period the grass available on the field will increase in maturity and so will be likely to decrease in digestibility. They proposed a cold storage method by which herbage feeding trials could be greatly simplified and errors due to changes in the composition of the herbage reduced.

Raymond et al. (1953a) described technique in which all the herbage required in a digestion experiment was cut and sampled at one time and then frozen and cold stored at 0 - 5° F until required. Raymond et al. (1953b) carried out experiments to test the assumption that the herbage fed after cold storage has the same digestibility as it would have had if fed fresh. The evidence obtained from their study showed that any effect of such cold storage on the digestibility of dry matter, organic matter, and nitrogen in herbage is small. Ekern et al. (1965) carried out experiments to determine whether changes in the nutritive value do occur when herbage is preserved in a frozen or dried state. Their results indicated that drying certainly, and freezing probably, increased the nutritive value of herbage as a source of energy by a small and statistically non-significant amount. They suggested that this effect was largely due to the smaller losses of heat and of CO_2 when metabolizable energy is derived from preserved herbage rather than from fresh herbage. Pigden et al. (1961) conducted experiments to study the comparative intake of fresh and frozen forages. The results indicated that sheep appeared to consume frozen forage direct from the freezer as readily as in the fresh state. The rate of dry matter digestibility of frozen forage showed some lag behind that of the fresh forage within the first 6 hours but this difference was overcome within 12 hours.

Factors Affecting in vivo Digestibility

Attempts have been made to study some of the factors that would influence digestibility values in vivo. Schneider and Lucas (1950) studied the magnitude of certain sources of variability in digestibility data. They found that the largest portions of error in digestibility

data are those associated with authors and samples. The portion associated with trial was found to be relatively small. Donefer (1966) found the major sources of experimental error to be due to differences in chemical composition of the forage caused by lack of uniformity of test material and to differences in the chemical and in vivo methods as used in the collaborating laboratories. Raymond (1951) discussed the factors likely to cause errors in measuring the nutritive value of herbage.

Raymond (1954) observed that of the factors that affect the digestibility of a given feed one that appeared to have been little studied was the possible variation in the digestive ability of ruminants with age. It was generally assumed that the digestive ability of a ruminant remained relatively constant after it was weaned. Raymond et al. (1954) investigated this problem using sheep ranging from lambs to 4-year olds. They found that digestibility increased linearly with age over the period from lambs to 2-year olds. The average increase in digestibility was about one unit for each year's increase in the age of sheep. Their data, however, did not show this to have occurred with all the feeds they used. Raymond et al. (1954) concluded that while the results did not justify the correction of digestibility data for age it seemed advisable to be aware of the trend indicated. Holmes et al. (1961) noted that there was evidence that calves selected a diet of higher digestibility than cows.

The effect of intake on digestibility has been investigated by many workers (Crampton, 1957; Blaxter et al., 1961; Blaxter and Wilson 1962; and Elliot and Tops, 1963). Blaxter and Wilson, 1962 reported significant differences between individuals in their voluntary food

intakes and those individuals which consumed most digested it least efficiently. The variation in intake from individual to individual expressed as a standard deviation was ± 7.5% of the mean. Donefer (1966) observed that standardized in vivo methods would seem to be warranted in order that the large differences noted in variability of intake as measured from among as compared to within--laboratory sources might be lessened. It is recognized that the measurement of voluntary intake can best be achieved when the mechanism controlling its regulation have been fully elaborated. Balch and Campling (1962) made an extensive review of regulation of voluntary food intakes in ruminants. Blaxter et al. (1956) recommended that in conducting digestibility trials frequent feeding at equal intervals is more desirable and a departure from such a regimen even for a single day will result in changes in the rate of feces production and hence errors in the weights of collections of the feces in subsequent days. A preliminary period of 10 days was considered sufficient to annul the effects of a previous ration, and to ensure that collections of feces are representative of the food given (Blaxter et al., 1956).

Balch et al. (1953) studied the effect of a restricted water intake on the digestibility of hay and they reported that it was unlikely that a restricted water intake produced any great change in the rate of passage of hay; although such differences as were found indicated a trend towards lower excretion. The results of this experiment suggested that when water intake was restricted adjustments took place in the water economy of the cow favoring the maintenance of a water to dry matter ratio in the reticulo-rumen similar to that present when the water was freely available (Balch et al., 1953).

Small Sample in vivo Technique

Conventional digestion trials with large animals are prohibitive for screening a number of forages or for routine forage testing programs because of the time and expense involved. Lusk et al. (1962) described the use of a small sample in vivo technique. Small samples of forage in nylon sacks were suspended in the rumen of fistulated cows to determine how the coefficients of cellulose digestibility obtained by this method compared with those obtained by conventional digestion trials. In this study Coastal Bermuda hay and alfalfa hay were used. They found no significant difference between the cellulose digestibility coefficient at the end of 72 hours by small-sample method and the cellulose digestibility coefficient obtained by conventional methods. A significant (positive) regression of $Y = 4.86 + 0.7947X$ ($r = 0.83$) was obtained where Y = small sample digestion (% for cellulose)

X = Digestion by conventional method (% for cellulose)

Lusk et al. (1962) noted that their results indicated that the small sample technique used with a regression equation might provide a valid estimate of cellulose digestion. Van Dyne and Meyer (1964a) described the use of a similar microdigestion technique for measuring forage intake of grazing livestock.

Pettyjohn et al. (1964) described a method which consisted of placing a feed stuff sample in a dialysis bag with rumen fluid, suspending the bag in a perforated plastic cylinder and incubating in the rumen of a fistulated animal. Pettyjohn et al. (1964) listed the following advantages of small sample in vivo methods.

1. Sample can be set up with minimum of exposure of rumen fluid to detrimental conditions.
2. The rumen fistulated animal provides normal rumen pH, temperature, digestion and end product removal.
3. It permits small sample size and facilitates comparing several forages simultaneously.
4. The nature of the bag maintains the integrity of the inoculum, prevents the entrance of large non-dialyzable molecules or particles and allows diffusion of end-products, e.g., volatile fatty acids without the escape of small feed particles.

Pettyjohn et al. (1964) suggested that methods such as this need testing with a large number of samples of varied forages with known digestibilities to determine the precision that can be attained and the validity of estimate between and within forage types.

The literature reviewed here seems to indicate that sheep and cattle possess similar digestive powers. It is possible that with more experiments and greater precision and accuracy in techniques, differences between these two species may be revealed in future. There are definite advantages in using sheep in digestion trials. Firstly, sheep are less expensive to maintain than cattle and secondly the investigator can use more animals per trial when sheep are used instead of cattle. The use of goats in digestion trials should be given more consideration particularly in African or Tropical countries in general. If it can be used and provide results similar to those obtained with cattle, then more digestibility information will be obtained on African forages. At the moment there is a critical shortage of this information as well as information on the factors

which affect the nutritive value of tropical forages. The use of fistulated animals represents a significant contribution to forage evaluation particularly in using animals as sampling agents.

As Donefer (1966) rightly pointed out, in order to be able to standardize in vivo methods more information is needed on the mechanisms involved in regulation of food intake.

As already pointed out the in vivo method has been criticized because small samples of forage could not be evaluated with its use. The small sample in vivo technique reviewed in this report represents, therefore, a significant innovation in the use of the live animal in forage evaluation. The reliability of results obtained by indirect methods of forage evaluation depends on how close such results are to the corresponding values obtained in vivo. Such a comparison will always be needed. The in vivo method, therefore, will continue to be an important technique in forage studies.

DIGESTION TECHNIQUES USING RUMEN MICROFLORA IN VITRO

In vivo digestibility trials are expensive and time consuming. Furthermore the in vivo method is not so readily applicable to small quantities of forage that are usually available from the plant breeder's nursery or from small plot agronomic experiments. Considerable interest has developed in the use of the in vitro rumen fermentation technique for the evaluation of forage quality (Barnes, 1965).

In the in vitro rumen fermentation technique, an attempt is made to duplicate the conditions existing in the rumen. Temperature is usually controlled at 39-40° C by means of a water bath, anaerobic conditions maintained by passing CO_2 gas through the test solution, acidity is controlled to near pH 7 by "artificial saliva" buffer system, bacteria obtained from the rumen are added and a forage source is provided (Van Dyne, 1962). Digestive activity is commonly evaluated by determining disappearance of e.g. cellulose or dry matter. Warner (1956) proposed the following requirements in developing procedures for in vitro evaluation of nutritional quality of forages.

1. The maintenance of normal appearance, numbers and proportions of the bacteria, selenomonads and protozoa.
2. The maintenance of normal rates of digestion of cellulose, starch and protein and the normal interaction between components.
3. The ability to predict in vivo results quantitatively from in vitro studies. Drew (1966) presented the following as some of the factors affecting the results obtained from in vitro studies:

1. Type of in vitro system.
2. Treatment of substrate prior to digestion.
3. Source and preparation of the inoculum.
4. Length of fermentation period.

Effect of Diet Fed to Inoculum Donor Animal

Reid et al. (1964) found that in vitro digestibility was higher with inoculum from sheep fed hay as compared to that from sheep fed fresh grass. Van Dyne (1962) reported that there was greater cellulose digestion in both the mixed range forage and the Solka floc in trials where the cow was fed alfalfa hay as compared to the trials where the cow was fed oat hay. A question arises with regard to the validity of using the inoculum from an animal fed on a different kind of feed than the one being evaluated (Van Dyne, 1962). Bezeau (1965) carried out experiments to gain further information on the importance of the ration fed to the donor animal in in vitro forage evaluation. Five hays of different quality were used as the source of both inoculum and substrate and digestible cellulose was used as the criterion for evaluating inocula. The standard deviations indicated that as a source of inoculum, the native prairie hay was significantly (P .05) more variable than the other hays. Duncan's multiple range test showed no significant difference between the three alfalfas as the source of inoculum. However digestion by alfalfa 1 and alfalfa 3 inocula were significantly greater than that from grass or native hay and inoculum from the grass hay was superior to that from native hay.

Work by Church and Peterson (1960) indicated that variability may be minimized by feeding the donor animal a ration similar to the

substrate. Knipfel and Troelsen (1966) reported that comparable results were obtained by feeding the inoculum donor a diet that covered the range of ingredients which were present in the substrate being analyzed. Bezeau (1965) observed that the digestibility was not significantly different when the inoculum was from the same hay or when the inoculum and substrate were from different hays. Troelsen and Hanel (1966) noted that the effect of inoculum donor on the digestibility of different fractions of the substrate was only slight ($P < .05$) and sporadic. Shelton and Reid (1960) reported that the degree of cellulose digestibility at a particular stage of maturity varied significantly according to whether the inoculum was taken from sheep receiving grass at the same stage of growth or from sheep fed a standard hay ration.

Knipfel and Troelsen (1966) made studies to determine the effect of similarity between the substrate and the diet of the inoculum donor, on the in vitro digestibility of dry matter and organic matter. The digestibility data revealed interactions among inoculum donor diets, in vitro substrate mixtures and fermentation periods. Knipfel and Troelsen (1966) noted indications that alfalfa possessed a specific growth promoting effect on the microflora. Kumeno et al. (1967) attempted to evaluate the possible influence of inoculum source on the in vitro dry matter digestibility using mixed feed substrate. Rumen inocula obtained from animals on either an all hay ration or a high concentrate ration were compared and no significant differences were observed between these two types of inoculum. Quicke et al. (1959) reported that the digestibility of cellulose in vitro in a given forage

was the same irrespective of the forage fed to the steer used as a source of inoculum.

Digestive Power of Inoculum from Sheep and Cattle

Inoculum from different sheep fed identical diets in the same environment may be expected to possess similar microbial activity (Troelsen and Hanel, 1966). Bezeau (1965) reported significant differences in the activity of inocula from the two donor steers he used. Drew (1966) reported that his results demonstrated the similarity of results of mean in vitro estimates of apparent digestibility obtained by using rumen liquor from either sheep or cattle provided they are fed the same diet. With the sample of each donor species used there was slightly less between animal variability for sheep than for cows (Drew, 1966). Van Dyne and Wier (1966) observed that little information was available concerning the variation of digestive power of the inocula from animals grazing under varying range conditions.

Length of In Vitro Fermentation Period

The length of the fermentation period in the artificial rumen technique is one of the factors that influence the digestibility values obtained by this method. Van Dyne (1962) reported an increase in cellulose digestion with increasing fermentation time periods irrespective of the source of cellulose. Barnes et al. (1964) found a linear relationship between length of fermentation and the magnitude of cellulose digestibility. Knipfel and Troelsen (1966) obtained comparable results by extending the period of in vitro digestion to 48 hours. Chalupa and Lee (1966) found that lag-time differences were

evident at 6, 12, and 18 hours whereas incubation beyond 24 hours was essentially a measure of total digestion. Coefficients of variation in in vitro cellulose digestibility were lower with longer fermentation periods. Karn et al. (1967) found that the repeatability of the cellulose digestion procedure increased as the fermentation time increased. The opposite seemed to be true with the dry matter disappearance procedure. Karn et al. (1967) stated that the measurement of the digestion occurring between the fermentation periods 5 and 8, and 8 and 11 hours should be optimum for the study of in vitro rate phenomenon.

Kumen et al. (1967) observed that the optimum conditions for the study of digestible energy of mixed rations would be a 48 hour fermentation period using 0.75 gram substrate. There was a highly significant correlation (r = 0.85) between dry matter digestibility in vivo and the 48 hour dry matter disappearance in vitro. IfkoVits et al. (1965) using the pure culture inoculum method reported that the 48 hour fermentation period gave maximum cellulose digestion. Troelsen and Hanel (1966) noted that whereas alfalfa had reached maximal digestion after 48 hours of fermentation, wheat straw had not reached the corresponding plateau at 96 hours. The review by Balch and Campling (1962) revealed that as forages become coarser and more mature, they could be expected to remain longer in the rumen exposed to digestion by microflora. These observations point to the need for extension of the in vitro fermentation procedure to include an estimation of the fermentation time required to attain a digestibility similar to that attainable in vivo (Troelsen and Hanel, 1966).

Effect of Washing Fistula Samples on in vitro Digestibility

Robards and Wilson (1967) examined the effect of washing on the in vitro digestibility of fistula samples and the results were compared with the digestibility of plant material cut from the pastures. Data from ungrazed plots showed that washing significantly lowered the in vitro digestibility value. The digestibility of washed samples from esophageal fistula was reduced by an average of 7.0 digestibility units. The work of Langlands (1966) showed that saliva can increase apparent digestibility by 1.6 units. Thus the remaining 5.4 digestibility units would be due to loss of soluble plant material and fine particles during washing. Van Dyne (1962) reported a greater than 10% difference (highly significant) in estimates of dry matter digestion by the two rinse procedures he used (these were: thoroughly rinsed and lightly rinsed). The corresponding differences between the two rinse procedures for cellulose digestion were highly significant although relatively small 3.8%. This indicated that under these circumstances, dry matter digestion was a much less accurate measure than the estimate of cellulose digestion. Robards and Wilson (1967) recommended that if an actual digestibility estimate for each forage species was required, an in vitro digestibility value could be corrected by the addition of five digestibility units. This recommendation, however, seems to be based on two questionable assumptions, namely that the digestibility of washed samples will always be lower by 7.0 digestibility units and secondly that saliva would increase apparent digestibility by 1.6 units in all cases.

Use of Pure Culture Inoculum in In Vitro Studies

Results have been reported on the use of the pure culture inoculum method in in vitro digestibility determination (Ifkovits et al., 1965 and Dehority and Scott, 1967). Ifkovits et al. (1965) obtained significant correlations between pure culture and mixed culture inoculum in vitro method. The following regression equation was derived:

$$Y = 6.60 + 0.89X$$

where Y = percent cellulose digested by mixed culture rumen inoculum

X = percent cellulose digested by B. succinogenes S85.

Regression of in vivo digestible dry matter (Y), on 48 hour in vitro cellulose digestion by S85 (X) gave a line which was described by:

$Y = 35.17 + 0.60 X$ (S.E. 3.29). The corresponding equation for in vivo digestible organic matter (Y) was

$Y = 42.60 + 0.54X$ (S.E. 2.94)

Dehority and Scott (1967) investigated the ability of 9 pure strains of rumen bacteria to digest the cellulose. It was found that for all organisms and forages, the amount of cellulose in a forage available for digestion by a specific organism decreased as the forage matured. It was also reported that alfalfa cellulose was less available to all organisms than grass cellulose.

Plant Breeder's Modified In Vitro Method

In plant breeding studies many small samples often taken from single plants need to be assessed for digestibility. A modified in vitro method for such estimations was described by Rogers and Whitmore (1966). The method described was successfully used for in vitro determinations

of the digestibility of clover, grasses, kale and alfalfa. Rogers and Whitmore (1966) proposed that any method developed for such estimations should (a) with a minimum of labor detect even fairly small differences in digestibility between samples of herbage, (b) also give good agreement between different assessments of the same sample. Relative rather than absolute determinations are important to the breeder (Rogers and Whitmore, 1966).

Use of In Vitro Digestibility Results to Estimate In Vivo Values

Barnes (1965) emphasized that a laboratory method for routine evaluation of forage quality must (1) be relatively simple in order to allow the rapid analysis of a large number of samples (2) produce results with a high degree of precision (3) give an accurate unbiased estimate of forage quality. Many research workers have attempted to derive equations from in vitro digestibility data which could be used to estimate the in vivo digestibility of forages. The aim is that if a reliable relationship were established it would save time and expense involved in in vivo determinations. Quickie et al. (1959) stated that results for 9 forages indicated that the digestibility in vitro of cellulose as measured in 48- and 60-hour fermentations did not differ significantly (P 0.05) from digestion coefficients obtained in sheep trials. Baumgardt et al. (1962a) and Baumgardt et al. (1962b) reported that percent cellulose digested in vitro was significantly correlated with TDN, digestible dry matter and digestible energy (calories per gram) and the digestion coefficient of energy determined in conventional digestibility trials. The digestible energy estimates from the artificial rumen method and the prediction equation obtained from the study showed

a coefficient of variation of 5.2% when compared with the animal digestible energy values. Johnson et al. (1964) found that in vitro cellulose digestion was generally highly correlated to all in vivo values for grasses.

Bowden and Church (1962) reported that correlations between in vitro dry matter digestibility and in vivo dry matter digestibility were highly significant. Bowden and Church (1962) pointed out that their results tended to indicate that in vitro digestibilities and crude protein contents of forages were useful tools only when differences between forages being examined were marked. Drew (1966) using data based on 51 observations obtained the following regression of the in vivo coefficient of digestibility of feed organic matter on the in vitro estimates

$$Y = 0.88 x + 11.0 \pm 2.1 \quad (2.7\%$$

The error associated with individual estimates became progressively larger as apparent digestibility fell (Drew 1966). Alexander and McGowan (1966) using data obtained from 18 grasses and legumes and 25 hays derived the following regression

$$Y = 0.97 x + 5.05$$

where Y= in vivo digestibility of organic matter

X = in vitro digestibility of organic matter

The coefficient of correlation, r, and the regression standard deviation of the above relationships were 0.96** and 2± units respectively.

Baumgardt et al. (1962a) attempted to use an artificial rumen method in which the fermentation of forage carbohydrate was measured. Their results yielded estimates of TDN significantly correlated with

the animal digestibility data. These TDN estimates, however, were consistently lower than the actual TDN values. The factors that tended to reduce the usefulness of this method were discussed by Baumgardt et al. (1962a).

Donefer et al. (1962) found the 12 hour in vitro cellulose digestion (X) to be highly correlated with the nutritive value index (NVI) - (Y) of 26 forages fed chopped (r = 0.96) and 16 forages fed ground (r = 0.87). Prediction equations for NVI (Y) of chopped and ground forages were respectively

$$Y = -3.5 + 1.23 X$$

$$Y = 7.4 + 1.23 X$$

The latter equation can be expressed as $Y = -3.4 + 1.23X + 10.9$ to illustrate the observed increase of 10.9 NVI units as a result of grinding of forage. Earlier on Donefer et al. (1960) had derived the equation

$$Y = 7.8 + 1.34 X$$

where Y = NVI

X = 12 hour in vitro cellulose digestion

Chalupa and Lee (1966) stated that digestible energy could be predicted accurately from in vitro cellulose digestion whereas indices including intake (Relative Intake, Nutritive Value Index) were more variable. They concluded that in vitro cellulose digestion could be used as a rapid means of screening forages for their energy availability. Chalupa and Lee (1966) suggested the use of 18-hour in vitro cellulose digestion in estimating NVI.

Karn et al. (1967) came to the conclusion that the rates of cellulose digestion and dry matter disappearance were not more highly correlated

with in vivo data than either a single cellulose or dry matter disappearance figure obtained at a given fermentation time. During digestion by rumen organisms much of the herbage is converted into bacterial protein, which would be digested by the animal further down the alimentary tract but remains as undigested residue in the in vitro procedure (Raymond 1966). Consequently it has been proposed that digestion in vitro by rumen organisms should be followed by a second digestion with acid pepsin. A detailed description of such a two-stage technique for in vitro digestion of forage crops has been given by Tilley and Terry (1963). Using this technique Tilley and Terry (1963) studied 148 herbages of known in vivo digestibility and obtained the following linear regression equation

$$Y = 0.99\ X - 1.01\ (S.E. \pm 2.31)$$

where Y = percent in vivo dry matter digestibility

X = percent in vitro dry matter digestibility

Tilley and Terry (1963) also reported that within an experiment the standard error of the mean duplicate estimates was ± 0.66 digestibility units and between experiments the standard error of the means was ±1.18. A close correlation (r = .97) was found between digestibilities measured in vivo and the two-stage in vitro technique.

Johnson et al. (1962) investigated the differences in nutritive value between grasses and alfalfa when estimated by in vitro techniques. Their results indicated that when only grasses were considered the correlation between the digestibility of dry matter, cellulose and energy and in vitro cellulose digestibility were very high and highly significant. Regression of the 12-hour in vitro cellulose digestibility of grasses on nutritive value indeces resulted in a line which

was described by Y = 1.087X - 15.94 (r = 0.95). When the data for alfalfa were included the new line was described by Y = 0.814X - 5.94 (r = 0.86) where

Y = 12 hour in vitro cellulose digestibility

X = nutritive value index.

It appeared that alfalfa did not fit the pattern established by grasses and when included in correlation analyses lowered the value of the comparisons (Johnson et al., 1962). Naga and el-Shazly (1963) reported a significant correlation (r = 0.902) between the in vitro cellulose digestion and the digestible energy per kilogram dry matter (DE/kg DM) in the case of non-leguminons roughages. The legumes showed no significant correlation in vitro even when the digestion was continued over 12, 18 or 30 hours and DE/kg DM. Naga and el Shazly (1963) discussed the probable reasons for this difference in behavior between legumes and non-legumes. It was concluded by Naga and el-Shazly (1963) that the use of artificial rumen gave the best criterion of the nutritive value of forages other than legumes and enabled the best approximation of energy to be arrived at.

Tomlin et al. (1965) found that lignin content was negatively correlated with in vitro cellulose digestibility at the 72-hour time period for grasses and legumes; although the regression equation for the groups were significantly different. Lignification was linearly related to cellulose digestibility as the grasses matured, however this relationship did not exist for alfalfa. Wilkins (1966) reported that the relationship between the digestibilities in vitro of organic matter and cellulose was close in case of grasses (r = +0.97, P 0.001) but it was

not significant for non-grasses. Wilkins (1966) further reported that the correlation between organic matter digestibility and nitrogen content was significant at P 0.05 for non-grasses but not significant for grasses (r = +0.38). Alexander and McGowan (1966) found no difference in the in vitro-in vivo relationship of digestible organic matter for grasses and legumes and that of hay.

Wilkins (1966) observed that the coefficient of variation for the determination of digestibility of individual samples was lower for organic matter digestibility than for cellulose digestibility. Troelsen and Hanel (1966) stated that digestible organic matter would be more descriptive than either cellulose or non-cellulosic organic matter digestibility for predicting the nutritional value of forages in general.

Johnson et al. (1964) commented that the decision as to which variable or combination of variables would be best to measure in order to predict most accurately the in vivo value of forages would depend on:

1. Whether the investigator wanted to study one type or class of forages e.g. all grasses or whether he was interested in all types including grasses and legumes.
2. Whether the investigator was willing to use a different equation for each class or needed one equation to fit all classes.
3. The coefficient of variation of the methods should also be considered.

The literature reviewed here tends to indicate, owing to the conflicting nature of the results, that the effects of source of inoculum on in vitro data need to be more clearly defined by further studies. Until adequate information is available it would seem to be better to feed the inoculum donor animal the same forage as the one the investigator intends to analyze in vitro. Owing to likely differences in degree of selective grazing by sheep and cattle or by different animals within each species, microbial populations of differing digestive ability may be developed. More studies therefore would help to evaluate the variation in digestive power of inocula from animals on pasture. The results examined here indicate that cattle and sheep would provide inoculum with similar digestive ability provided they are fed the same diet.

In general an increase in the length of the in vitro fermentation period results in an increase in digestibility. Depending on the type of forage and the digestibility coefficient(s) used to evaluate it, each investigator may have to determine the optimum in vitro fermentation period under given set of circumstances. When a number of experiments have been run using certain forages, it is possible that standard fermentation periods will be established for these. The pure culture inoculum method would be criticized on the ground that it sacrifices the possible synergisms between different rumen micro-organisms whereas the mixed culture inoculum utilizes them.

One of the limitations of regression equations derived for the estimation of in vivo nutritive value of forages is that for a given forage a new equation has to be calculated for each locality.

Furthermore, the inherent variability of biological material presents a serious limitation when one tries to use a mathematical relationship to describe or predict the nutritive value of a feed. Even within the same locality and the same forage, there will be a season-to-season variation in the nutritive value of a forage. These variations may be due to change in weather conditions or treatments applied to the forage e.g. fertilizer application. The nature and amount of fertilizer applied would be an important factor. In spite of these limitations mathematical relationships as those reviewed here serve a useful function as a quick means of evaluating forages and the estimates thus obtained are useful guides on the nutritive value of a forage. It is important to emphasize that these estimates are only guides, the final measure of a feed is its effect on the ruminant.

PREDICTION OF FORAGE VALUE FROM CHEMICAL ANALYSES

Marble (1965) stated that the motivating reasons behind the increased interest in forage evaluation by chemical analyses were: more accurate methods of evaluation, development of good research data indicating how quality forages can be produced, a cost squeeze that has produced more efficient farmers who, in turn, recognize the value of better quality forages and a greater awareness by extension workers of the value of forage testing and promotion of forage testing programs.

Conventional Method of Proximate Analysis

The Weende's system of proximate analysis has been in use for over a hundred years. This scheme of analysis was devised by workers at the Weende Experiment Station in Germany. According to it a feed stuff is partitioned into six fractions as follows:

Water

Ether Extract (EE)

Crude Fiber (CF)

Nitrogen--Free Extract (NFE)

Crude Protein (CP)

Ash

Only five of these fractions are actually determined, the NFE is determined by difference.

A survey of the literature reveals considerable dissatisfaction by many workers with the Weende system of feed analysis particularly

with the crude fiber method and calculation of NFE (Hallsworth, 1950; Hansen, et al., 1958; Walker and Hepburn, 1959; Kivimae, 1959; Fischer, 1961a, Fischer, 1961b; Sullivan, 1962; Van Soest, 1963a; Van Soest, 1966; Moore, 1966; and Van Soest, 1967). Neither the crude fiber nor the NFE fractions represent discrete chemical entities but are highly complex and variable (Hansen et al., 1958). The NFE comprises a fairly heterogeneous group of compounds, mainly carbohydrates and similar substances which are not accounted for in the direct fodder analysis. The CF fraction has also been criticized as an emperical value (Walker and Hepburn, 1959 and Gaillard, 1966) which combines digestible and indigestible parts of the food. The CF is thus a heterogeneous substance consisting mainly of cellulose and lignin and a small amount of protein (Kivimae, 1959). Kivimae (1959) further noted that the relationship between these components did not only vary from one plant species to another, but also from one sample to another during the course of growth.

The division of the carbohydrate fraction of the feedstuff into CF--supposedly the indigestible and insoluble fractions--and the NFE--supposedly highly digestible is not a truly realistic nutritional seperation (Moore, 1966) because the separation does not accomplish what was intended. Moore (1966) presented data which showed that in a certain study, in 30% of the cases of dry feed the CF or insoluble fraction was as digestible as the NFE or soluble fraction. Sullivan (1962) stated the CF does not necessarily represent the less digestible portion and that NFE does not have the high digestibility often attributed to it. The principal reason for such findings is that lignin,

an indigestible constituent of feeds, is partly soluble in weak alkali and, therefore, goes into the NFE fraction (Moore, 1966). These observations, therefore, challenge the conceptual basis on which the proximate analysis scheme was originally advanced.

Sullivan (1962) in a critique of evaluation of forage crops by chemical analysis, seriously questioned the necessity of determining all the components indicated in the proximate scheme. For example, he considered that the determination of ether extract was not accurate and as the total quantity in most forages was low, this fraction was of minor importance. Although the need for better chemical methods of feed analysis has been recognized for a long time (Kivimae, 1959 and Van Soest, 1964), feed analysts have been reluctant or slow to abandon the Weende scheme. Hallsworth (1950) observed that some of the reasons why CF determination has been retained in spite of the criticisms raised against it were that a considerable number of analyses that had been made in the past served conveniently for comparison and secondly statistical analysis of composition and digestibility of feeding stuffs data showed that CF was significantly related to digestibility. However Van Soest (1966) quoting Paloheimo (1953), wrote that the retention of CF has been related partly to misconceptions about its nature and partly to lack of understanding of the biochemical factors influencing the nutritive availability of different chemical fractions in feeds. In recent years, attempts have been started to find a scheme of feed analysis that would yield nutritionally meaningful fractions and thus eventually replace the Weende method. These studies will be reviewed later in this section.

Relationship Between Chemical Composition and Digestibility

Various attempts have been made to develop mathematical relationships between digestibility of feeds and one or more of the chemical components of these feeds. The main objective has been to avoid the laborious, expensive and time consuming in vivo experiments since chemical analyses can easily be carried out on a routine basis. Expressing a similar opinion Armstrong et al. (1964) suggested from the results of their study that for advisory purposes the additional time and expense involved in measuring apparent digestibility was not worthwhile; simple chemical analyses of a representative sample would enable a slightly less precise estimate to be obtained in a much shorter time. Progress in this field was reviewed by Miller (1961).

(a) Crude Protein:

The crude protein content of forage has been used as an indicator for estimating digestibility since protein is relatively easy to determine and a positive relationship between protein content, and digestibility has been shown (Axelsson, 1952; Hawking, 1959; and Kivimae, 1959; Hutton, 1962; Minson and Milford, 1966). Sosulski and Patterson (1961) investigated the relationship between the proximate analysis and lignin content of certain grass strains and species and their digestibility by sheep. They found that the most digestible forage was significantly lower in crude fiber and lignin and significantly higher in protein. Stallcup and Davis (1965) reported a highly significant correlation ($r = 0.95$) between crude protein content of forages and digestible protein. Such a high degree of relationship makes the crude protein determination of forage dry matter a very valuable deter-

mination in so far as prediction of nutritive value of a forage is concerned (Stallcup and Davis, 1965). Earlier, Axelsson (1950) had found that the protein content influenced the digestion coefficient of the protein more than the coefficients of the other nutrients.

Holten and Reid (1959) observed that for green forages there was a highly significant curvilinear relationship between apparent digestibility of protein and the concentration of crude protein in the forages. They obtained the following expression using green forages fed to cattle:

$$Y = 34.90\ (X - 5)^{0.297}$$

where Y = apparent digestibility of protein (%)

X = crude protein content (%)

It was found that the equation of the general form $Y = a + b + c \log X$ provided an excellent fit to the data for all classes of forage included in their study. It was suggested that this was the result of the decrease in relative contribution of metabolic nitrogen to fecal nitrogen as the crude protein concentration in forages increased. Glover et al. (1957) obtained between the digestibility of protein (y) and protein content (x) the following equation $y = 70 \log x - 5$. According to this equation the digestibility of protein increased very rapidly at low protein levels (between 2 and 8%) and more slowly as the content increased over 15%.

The use of crude protein as an indicator of forage digestibility has some disadvantages (Kivimae, 1959) of which the variations in the protein quality and variations in the endogenous protein content in the feces are the most important. Armstrong et al. (1964) also commented that crude protein variations caused by variation in botanical composition

of herbage were particularly difficult to assess in mixed herbages. Van Soest (1967) noted that crude protein was not likely to be a very reliable predictor because it is much affected by nitrogen fertilization and relative differences in content among legume and grass species. Nevertheless, Sullivan (1962) stated that as the digestion coefficient for crude protein is positively and significantly correlated with the percentage of crude protein the latter does very well as a measure of quality.

(b) Crude Fiber:

Crude fiber is another feed component which has been used to evaluate forages. As Kivimae (1959) noted of the plant composition constituents crude fiber has been the one most commonly used as an indicator for estimating digestibility.

The evaluation is based on a negative correlation between crude fiber content and digestibility of a forage. Numerous workers have presented data to show that such a relationship exists and it is significant (Phillips and Laughlin, 1949; Axelsson, 1950; Hallsworth, 1950; Axelsson, 1952; Hawkings, 1959; Kivimae, 1959; Meyer and Lofgreen, 1959; Gaillard, 1962; Gangstad, 1964; and Stallcup and Davis, 1965). As it was indicated above, in recent years there has been a growing tendency among researchers to question the value of the crude fiber fraction as a measure of nutritional value of forages. The crude fiber fraction varies considerably and Kivimae (1959) reported that the amount and composition of crude fiber also depended on etiological factors (climate, environment, soil fertility) of which our knowledge is still scanty. Kivimae (1959) gave references to studies where contradictory evidence had been presented on the relationship between the

digestibility and crude fiber of forage crops. Van Soest (1964) pointed out that the modern criticism is not based so much upon the use of crude fiber to estimate nutritive value as it is upon the archaic character of the chemical isolation and the variable composition of the product.

(c) Cellulose and Hemicellulose:

Recognition of the limitations of using crude fiber to estimate nutritive value has led some research workers to use less variable (composition-wise) and nutritionally more realistic fractions. Suggestions have been made that the partitioning of the carbohydrate portion into lignin, cellulose and other carbohydrates may be of greater usefulness in predicting feeding value than the Weende division.

Burdick and Sullivan (1963) reported that the ease of solubilization and/or hydrolysis of the hemicelluloses was positively correlated with the digestion coefficient of dry matter in a number of forages including grasses and legumes. Sullivan (1966) analyzed 100 forage samples of known digestibility for hemicellulose, cellulose and lignin. He found that the hemicelluloses were lower in the legumes than in the grasses when expressed either as percent of dry matter or in relation to cellulose. There was a significant (P .01) correlation between the percent lignin and the percent of hemicellulose in grasses and alfalfa. Sullivan (1966) also observed that the quantity of either carbohydrate was not highly nor consistently correlated with digestibility and he noted that this was a matter of interest because the quantity of fibrous constituents is often used as a criterion of quality. The hemicellulose: lignin ratio was found to be significantly and negatively correlated with digestibility. Sullivan (1966) also discussed how the relationship

between hemicellulose, cellulose and lignin affected the digestibility of cellulose in grasses and alfalfa.

A survey of recent studies indicates an awareness that hemicelluloses are a more important factor in forage evaluation than was originally thought and as Van Soest (1964) commented the hemicellulose fraction remains one of the major problem areas. Hansen et al. (1958) noted that the lack of information regarding the constitution and properties of the hemicellulose could be ascribed to:

a) the lack of sufficiently good method for unequivocal separation.

b) the loss of identity of the isolated hemicelluloses.

c) differences in composition of the hemicelluloses extracted from different plants.

d) the fact that the polysaccharides isolated may constitute only fragments of the original polysaccharides.

e) the fact that hemicellulose fractions investigated by different workers have not always been comparable.

(d) Lignin:

Of the newer methods to replace in part the proximate scheme a promising one is that of lignin (Sullivan, 1962). Numerous authors have found significant negative correlations between lignin percentages and the digestion coefficients of dry matter, or oganic matter, and of energy and particularly of cell-wall substances such as cellulose (Meyer and Lofgreen, 1956; Kivimae, 1959; Sosulski and Patterson, 1961; Gaillard, 1962; Armstrong et al., 1964; Tomlin et al., 1965 and Sullivan, 1966). Sosulski and Patterson (1961) concluded that lignin was the

single best measure of apparent digestibility. However, Quicke and Bentley (1958) concluded from their study that in mature brome grass and orchard grass hays the differences in lignin content were too small to account for the observed differences in the digestibility of cellulose.

Van Soest (1964) commented that the manner in which lignin affected digestibility was not completely understood. A number of theoretical possibilities exist (Van Soest, 1964) and among these the traditionally popular one is that lignin inhibits digestibility because it encrusts the nutrients in the feed. Van Soest (1964) remarked that increases in in vitro digestibility of cellulose upon ball-milling (Dehority and Johnson, 1960) may mean either chemical degradation of the macromolecular structure or removal of encrusting lignin. More work was badly needed on the physiochemical relation of lignin and other plant constituents (Van Soest, 1964). After reviewing 151 papers on the general subject of lignin and feedstuff evaluation, Fischer (1961a) observed that the question of lignin digestibility was by no means settled.

Fischer (1961b) reviewed the various methods which had been suggested for the determination of lignin and he also presented a modified method of lignin determination. Recently, Czerkawski (1967) also described a modified method of lignin determination. One of the major problems in lignin determination is the efficient separation of protein from lignin without dissolving the latter. Van Soest (1964) discussed the causes of error in lignin determination e.g. non-enzymic browning due to heating. This artifact browning increases the amount of lignin quantitatively determined.

An interesting observation which has been reported in the literature is that alfalfa or legumes in general have a higher lignin content than grasses of equal digestibility (Kivimae, 1959; Van Soest, 1964; Van Soest, 1965;and Colburn and Evans, 1967). Gaillard (1962) also reported that it did not follow that the most lignified plant always showed the poorest digestibility. This observation has been explained by Van Soest (1964) thus: relative to grasses, alfalfa contains a smaller but more highly lignified holocellulose fraction that is considerably less digestible. In other words, this leaves a greater proportion of the dry matter of alfalfa independent of lignin. Since cellulose contents of alfalfa and grass are about equal, the principal species difference lies in the proportion of hemicellulose present which is higher in grasses than in alfalfa (Sullivan, 1966). Van Soest (1965) noted that the interrelationships between intake, digestibility and chemical composition were highly species oriented. He also called for the need to revise the old concept that fiber or fiber components and lignin were necessarily closely related and that both of these increased uniformly as the forage matured. Sullivan (1962) discussed the reasons why published regression equations for prediction of digestibility from percentage of lignin did not agree with one another.

(e) Methoxyl Content:

The quantitative determination of lignin in herbage is a very expensive and time consuming procedure. Some workers have proposed the indirect method of lignin determination by which some characteristic group of the lignin might serve as a measure of lignin content. The methoxyl content has been used by Richards and Reid (1952) and Anthony

and Reid (1958). Anthony and Reid (1958) derived the following regression equation:

$$Y = 91.01 - 13.968X$$

where Y = dry matter digestibility (%)

X = methonyl content (%) of forage dry matter

The standard deviation for the regression coefficient was 2.755 and the correlation coefficient for the relationship was -.828. These workers concluded that for the forages studied methoxyl content was associated closely with feeding value. They also added that methoxyl content of forage appeared to be useful as a relative index of forage digestibility for selecting among similar forages of widely different digestibility. Kivimae and Valdama (1964) reported their studies on timothy and proposed methoxyl as an alternative substance to lignin as an index of the nutritive value of timothy and similar grasses, because it was simple and inexpensive to determine and had a high repeatability.

(f) Multiple Regression Approach:

In reality digestibility is influenced by all the chemical constituents. Kivimae (1959) calculated multiple regression equations to investigate if the digestibility calculated by two or three reference substances yielded a better estimation of digestibility i.e. gave a lower standard deviation from the regression than did one variable. He found that the accuracy of estimation of digestibility increased considerably when both crude fiber and lignin were used in the same equation.

Minson and Kemp (1961) using data from 291 digestion trials derived the following equations:

$$D' = 59.7 + 5.20N_H \pm 6.17$$

$$D = 49.2 + 7.25N_F \pm 3.99$$

where D = organic matter digestibility of grasses

N_H = nitrogen percentage of the herbage dry matter.

N_F = nitrogen percentage of the feces organic matter.

The errors associated with the equations were high and it was found that the deviations from the lines were not randomly distributed but had a seasonal trend. By deriving multiple regression equations in which month of cutting was introduced, these errors were reduced by 1.15 and 0.53 digestibility units respectively. Sosulski and Patterson (1961) also reported that the multiple regression of digestibility of energy on lignin and protein proved to be a better measure of digestibility than lignin alone.

Gaillard (1966) using the multiple regression approach derived the following equation:

$$D.O.M. = -5.51 (L - 5.58) + 0.37 (C - 19.19) - 0.51 (H - 18.10 + 4.11 (U - 3.80) + 6.51$$

where D.O.M. = percent digestible organic matter

L = lignin (percent of roughage dry matter)

C = cellulose (percent of roughage dry matter)

H = hemicellulose (percent of roughage dry matter)

U = anhydryo-uronic acid (percent of roughage dry matter)

He pointed out that the above equation could probably be used for roughages of different origin and with a wide variety of digestibility as the material from which it was derived showed these two characteristics. More information, however, would be required to verify this claim. From his study, Gaillard (1966) concluded that the separate determination of lignin, cellulose and hemicellulose was needed for an accurate calculation of digestibility of organic matter of a roughage.

(g) Total Digestible Nutrients and Net Energy Systems:

The total digestible nutrients (TDN) system has been used for a long time to express the feeding value of feedstuffs. The TDN concept is based on the proximate analysis scheme and, consequently, any defects in the latter would affect the conceptual validity of TDN.

Axelsson (1952) made an extensive study of the relationships between contents of metabolizable energy, TDN, Scandinavian feed units, and starch units in the feedstuff. He found from his studies that the poorest feeds were overestimated and the best feeds underestimated in the TDN system. Maynard (1953) in a review article, attempted to clarify the source of misunderstanding in the use of TDN as a measure of feed energy. Stallcup and Davis (1965) concluded from their study that in the case of predictive regressions for TDN, values that have been derived from data on forages in a given area were more accurate than those derived from large numbers of observations from diverse areas or from forages of some other areas.

Blaxter (1956) stated that the TDN system measured what food contained rather than what animal performance it could promote and this was its weakness. Swift (1956) reported that a study of the relationship between digestible energy and TDN revealed a high correlation

coefficient between these two items (r = 0.97). Using figures from Morrison's Feeds and Feeding (1959), Moore (1966) showed that TDN and estimated net energy values for the concentrates generally agreed with each other; but as the TDN or energy content of the feeds decreased there was an increasing divergence (100 to 24.6%) in the values.

Lately there have been increased attempts to express forage value in terms of net energy. The net energy values take into account all the possible losses of energy in the feedstuff during its digestion and absorption. The net energy system is, on this account, considered to be a more accurate measure of the real worth of a feedstuff. Moore (1966) put it more bluntly when he wrote that net energy was the only valid method for expressing the energy value of feeds.

Moore (1966) attempted to answer the arguments which have been advanced against the use of the net energy system. For example it has been argued that there is a lack of sufficient number of determined energy values of feeds. This he answered by proposing the use of the following formula to convert the available TDN values into net energy values.

$$Y = 1.45X - 38.83$$

where Y = Net energy (NE)

X = Total digestible nutrients (TDN).

Thus approximate NE values could be calculated from TDN value of the feed which would be useful for practical feeding purposes (Moore, 1966).

Development of New Methods

Dr. P. J. Van Soest of the U.S.D.A. - Agricultural Research Services, has been responsible for the development of new methods and concepts in regard to the chemical evaluation of feeds. The division of the dry matter of feedstuffs according to the new method is shown in Table 1.

Using this new approach, Van Soest (1966) reported that higher relationships with digestible dry matter and organic matter were ($r = 0.79$) obtained with acid detergent fiber (ADF) than with the conventional crude fiber ($r = 0.73$). The correlation of the new lignin method and digestibility was -0.90 when grass and legume species were separated. In the development of this new method, emphasis was placed on the purity of the prepared fraction in terms of chemical entities, higher predictability of nutritive value, speed, economy and versatility in application. Kim et al. (1967) studied the qualitative and quantitative differences between the fibers isolated by the conventional method and the ADF method. They found that ADF contained more lignin but less pentosans and celluloses than the fiber isolated by the conventional method. Colburn and Evans (1967) derived regression equations for prediction of lignin and cellulose from ADF. The equations for alfalfa are shown below:

$$C = 4.56 + 0.81\ c \quad \text{(coefficient of variation = 1.75\%)}$$

$$L = 0.80 + 0.60\ l \quad \text{(coefficent of variation = 13.17\%)}$$

where C = predicted cellulose

c = percent ADF minus percent lignin

L = predicted lignin

l = percent ADF minus percent cellulose.

TABLE 1. Division of Forage Organic Matter by Systems of Analysis Using Detergents.

Fraction	Components	Nutritional Availability	
		Ruminant	Non-Ruminant
		Category A	
Cell contents (soluble in neutral detergent)	Lipids	Virtually complete	Highly available
	Sugars, organic acids and water soluble matter	Virtually complete	Highly available
	Starch	Virtually complete	Highly available
	Non-protein nitrogen	Virtually complete	Highly available
	Soluble protein	Virtually complete	Highly available
	Pectin	Virtually complete	Highly available
		Category B	
Cell-wall constituents (fiber in-soluble in neutral detergent)	Attached protein	Complete	High
Soluble in acid detergent	Hemicellulose	Partial	Very low
Insoluble in acid detergent (acid detergent fiber)	Cellulose	Partial	Very low
	Lignin	Indigestible	Indigestible
	Lignified nitrogen components	Indigestible	Indigestible
	Heat-damaged protein	Indigestible	Indigestible
	Keratin	Indigestible	Indigestible
	Silica	Indigestible	Indigestible

Source: Van Soest (1966)

Oh et al. (1966) found that acid detergent lignin (ADL) was more highly correlated with in vivo dry matter than was ADF or protein, especially when considered within species.

Wöhlbier (1965) expressed the opinion that "instead of wasting the benefits and the experiences obtained from costly experiments and efforts of so many highly qualified scientists on inventing new feeding systems, let us concentrate on improving our knowledge of energy conversions." The author of this report would disagree with the above opinion. It seems unrealistic to reduce the problem of feed evaluation to a matter of either this approach or none. The approach advocated by Wöhlbier (1965) is obviously required but the development of more refined and accurate feeding systems is equally urgently needed. The animal and its feed are in a sense interrelated. Information on the animal's feed requirements and the factors affecting its utilization is needed. Similarly, in order that the animal's requirements be met, accurate information on the nutritive value of the feed is essential. In short both approaches are needed. In any given case the determining factors should be the facilities available, area of specialization and interest of the research worker(s) concerned.

There is evidence in the literature of a growing criticism of and dissatisfaction with the continued use of the proximate analysis scheme as it has been used for the last hundred years. The most serious objection against the scheme is the variable nature of the CF and NFE fractions. This has made any comparison of forage on this basis to be viewed with suspicion. There is no doubt that as the precision and accuracy of the new methods improve, then the Weende scheme will have

either to be modified or completely replaced by a new scheme. The separate determination of lignin, cellulose and hemicellulose may become a regular feature in future forage analyses. This comment is based on the observation from the literature that the relationships between these entities offer a more realistic and meaningful explanation of differences found in nutritive value of forages than the CF fraction.

Given the fact that the nutritive value of forages is influenced by many, if not all, of the chemical constituents of the forages and also by environmental factors, a multiple regression analysis approach may yield valuable information. In trying to use the results of chemical analyses to estimate forage quality the same limitation mentioned in an earlier section is encountered, namely, the variable composition of biological material--forage in this case. Nevertheless chemical analyses serve a useful function in enabling a quick estimate of forage value to be made and this is very necessary under practical farm conditions. With the improving analytical techniques and advanced methods of data processing, chemical analyses will continue to be an important method in forage evaluation.

FORAGE EVALUATION FROM:
a) COMPARATIVE FEEDING TRIALS
b) RUMEN CONCENTRATION OF VOLATILE FATTY ACIDS

(a) Comparative Feeding Trial:

Mott (1959) stated that the response of the animal to the herbage consumed was considered by most investigators to be the most reliable measure of forage quality. Baily (1964) commented that as far as the ruminant was concerned, the definition of pasture quality in terms of chemical constituents in the herbage was still largely an unresolved problem and he went on to say that quality is best assessed on the basis of responses such as live weight gain in the grazing animal. For this reason it is considered desirable that chemical investigations aimed at defining quality in the herbage should use materials from pastures on which animal production is being measured so that the analytical results can be interpreted in the light of animal responses.

Many research workers have, therefore, attempted to measure forage quality by using, as a basis, live weight gain (Willhite et al., 1954; McCullough, 1963; Ingalls et al., 1965a, b; Milford and Minson, 1966; and Stobbs and Joblin, 1966a, b) and milk production (Cox et al., 1956; Brundage and Sweetman, 1962; and Browning and Lusk, 1966). Willhite et al. (1954) found a direct relationship between daily gain and crude protein available ($r = 0.95$). McCullough (1963) derived the following multiple regression equation:

$$Y = -13.633 + 8.228X_1 + .0004X_2 + .019X_3$$

where Y = average daily gain

X_1 = log of digestible dry matter

X_2 = body weight

X_3 = daily intake of digestible dry matter

He noted that the variables explained 67% of the variation in individual daily gains and that the equation was quite accurate for calculating the mean gains for the forages. Milford and Minson (1966) concluded from their study that because of the inadequacy of present methods of estimating the herbage intake of grazing animals, measurement of body energy gains was not justified and empty live weight gains were sufficiently precise to measure relative energy retention at pasture.

Cox et al. (1956) discussed the problems encountered in attempting to measure the output of grassland in terms of milk production. Many problems arise from the fact that two highly variable interacting biological systems, the lactating cow and the growing sward, are involved. Brundage and Sweetman (1960) found that digestibility was related to milk production.

Animal performances in terms of gain or milk production, obtained from various forages could result from differences in two interrelated aspects of ruminant digestion. These are:

(a) the rate of consumption of food.

(b) The efficiency of utilization of energy in the feed.

The first aspect involves the complex questions of voluntary intake and palatability of herbage. The regulation of voluntary food intake in ruminants was extensively reviewed by Balch and Campling (1962). They

came to the conclusion that several factors could affect the voluntary intake of food by ruminants, with different factors limiting in different situatiohs and they noted that this area required further investigation. Results of studies on palatability of herbage and animal preference have been summarized in reviews by Ivins (1955), Garner (1963) and Heady (1964).

Research workers have been interested, as a matter of practical information, in finding out whether animal preference for a forage could be used as a reliable measure of the nutritive value of that forage. Gangstad (1964 and 1966) noted that the high correlation of palatability to potassium content reflected the preference of the grazing animal for the more tender, succulent and turgid plant tissue of one variety as compared to another. He further observed that selective grazing of varieties was positively correlated with crude protein, percent moisture, percent juice and percent total sugar. Bryant et al. (1966) compared two silages on the basis of the stage of maturity and they found from the palatability trials that when the silages were fed simultaneously (in separate mangers) a marked preference for mature silages was shown by the animals.

As Gangstad (1964) pointed out, palatability in forage is a difficult and elusive characteristic to measure because it is known to vary with most of the factors and conditions which affect growth and development. Moreover, objective measurement is frequently limited to a subjective definition i.e. preference of the grazing animal which reduces objective data to a set of emperical values.

In reading the reviews referred to above on the palatability of herbage it becomes evident that information on this subject is conflicting. For example, some workers reported relationships between chemical composition and palatability, while others could not find any relationships between them. It has also been observed that a certain forage when offered for the first time to the animals may be rejected while the same forage will be readily consumed later when the animals get used to it. This then would lead one to question the validity of rejecting a forage on the basis of palatability results in, say, a plant breeding program.

Furthermore, in his review Garner (1963) quoted studies which had indicated that different forages were ranked differently at various times of the year i.e. a particular forage was not consistently preferred by the animals throughout the year. This would further suggest that there is no common factor which decides which plants are eaten and, consequently, as Garner (1963) proposed management should be so arranged as to produce a mixed flora as this would give better results in the long run. Results of palatability studies have also been questioned on the ground that in these studies research workers have tended to avoid the following classes of stock: very young stock, those lactating and those approaching the final stages of fattening, because they very easily go off feed.

In cases where a relationship has been found between palatability and say, crude protein, it would be interesting to investigate what happens when forages of the same crude protein content are offered. Future palatability studies should include a number of factors at a time instead of concentrating on single factors or even more simply, just

recording the magnitude of preference. The animal and plant factors affecting palatability and animal preference need further elucidation before these concepts can be used with any degree of certainty in forage evaluation.

(b) Rumen Concentration of Volatile Fatty Acids:

Rice et al. (1962) noted that the discrepancy between conventional digestion trial evaluation and actual forage feeding value may be related to the nature of the rumen fermentation. An evaluation based on rumen bacterial fermentation (Rice et al., 1962) may provide a more accurate measure of its feeding value. Naga and el-Shazly (1963) commented that a satisfactory comparison of forages must reflect the contribution made by all the constituents undergoing fermentation to supply energy. It was suggested, therefore, that the total volatile fatty acids (VFA) or organic acids produced in vitro may provide a better criterion since they reflect the fermentation products from carbohydrates, proteins and fats. Gray et al. (1967) stated that knowledge of the amounts of VFA produced together with the amounts of protein reaching the abomasum, should be a useful basis for comparing the nutritive values of different fodders. There is, therefore, increasing interest in investigating the possibility of using VFA production as a means of forage evaluation.

Asplund et al. (1958) found that total acid production was appreciably higher for inocula from sheep fed hay than for those from sheep fed straw. The coefficients of correlation between both VFA production and dry matter loss in vitro and dry matter digestibility in vivo were of the order 0.7 - 0.8 and either significant or highly

significant. Rice et al. (1962) found that the use of alfalfa inoculum or alfalfa substrate resulted in significantly (P .05) greater production of VFA than either oat straw inoculum or substrate. They observed that after 24 hours of in vitro fermentation there were no significant differences in the ratios of acetic acid to propionic acid. These results tended to agree with those of Warner (1956) who concluded that after 8 hours in vitro rumen fermentations no longer reflect the conditions existing in vivo.

el-Shazly et al. (1963) found a highly significant correlation (r = 0.819) between VFA production and digestible energy per kilogram of dry matter in all the roughages they studied. A similar value (r = 0.839) was obtained when organic acids (OA) was used as an alternative to VFA. They concluded from their studies that the in vitro production of (OA) or VFA gave a more accurate basis than in vitro cellulose digestion for predicting digestible energy per kilogram of dry matter. There is still uncertainty as to the exact quantiative contribution of the acids to the energy nutrition in ruminant animals (Rook, 1964). Gray et al. (1967) reported that in their study the energy of the VFA produced in the rumen was equivalent to about 54% of the digestible energy of the diet.

Bath and Rook (1965) observed that the molar proportions of the VFA in rumen liquor obtained with a given diet varied from animal to animal and that within one class of foodstuff important differences from food to food also occurred. Nevertheless broad generalizations could be made about the patterns of fermentation characteristic of different classes of foodstuffs. Raymond (1966) reviewed some of the

studies that have been made on the patterns of VFA production and he noted that the reasons for the changes in the proportions of acids in response to the use of different feeds or different levels of feeding were not known. Bath and Rook (1963) stated that the relative importance of the various factors affecting VFA production in the rumen certainly needed clarification.

Rook (1964) noted that because of the continuous production and absorption of the acids, and the onward passage of digesta the measurement of the ruminal production of VFA has proved technically difficult. He presented a brief description of the various approaches that had been devised to measure VFA production. Warner (1964) made a critical and extensive review of the methods of measuring the volatile fatty acids produced in the rumen. Kingsbury (1965) commented that because of many variables, there are difficulties in interpreting levels of VFA in samples from the rumen. He briefly discussed these difficulties. He further noted that if this technique could predict and give valid comparisons of energy available to grazing animals, it could be of value to plant breeders--that is--it may be used to predict the strains or species of pasture from which the grazing animal will ingest the most energy. Bath and Rook (1965) stated that the information they obtained could be of limited use as a basis for judging the likely pattern of ruminal fermentation of mixed diets given under ordinary farm conditions. This was so because the results were obtained from too small a number of samples and test cows to be considered wholly typical of other foods and animals. Furthermore the proportions of acids found when a food is given singly is only a rough guide to the effect that it will have on fermentation end products when included in a mixed diet.

Blaxter (1962) in discussing the utilization of the energy of the end-products of the digestion process, specifically discussed the utilization of the VFA. The use of VFA concentration as a means of a forage evaluation is a nutritionally valuable approach. The method will be particularly useful when the factors affecting the pattern of production of VFA with various feeds have been clarified.

SUMMARY

The aim of this report was to summarize some of the results of the work which has been carried out concerning the various methods of forage evaluation. The indicator technique saves a considerable amount of time and labor involved in total collection of feces and measuring amount of forage consumed both of which must be done in the conventional digestion trial. Of the external indicators chromium oxide has been used most. There are, however, two limitations to the use of chromium oxide and these are low degree of recovery of the indicator and the marked variability of its excretion pattern. No satisfactory reason has been proposed to account for this low degree of recovery. The method of sampling feces to obtain a representative sample for marker determination requires more investigation plus the related problem of factors influencing the excretion pattern of chromium oxide.

Some reports indicated that the digestive ability of sheep and cattle is the same, while others pointed out evidence to the contrary. More studies on this matter would be of value. Digestibility results obtained from animals with harnesses are usually applied to the larger population of animals under normal conditions. In view of this, more studies on the performance of animals with harnesses compared to those without harnesses may yield valuable information. Fistulated animals are playing a very important role in forage studies. As Robards and Wilson (1967) commented the material separated out from fistula samples is the most accurate sample that can be obtained of the species eaten.

The effect of age of the animal on in vivo digestibility of forage has not been studied to any extent. The few reports reviewed here indicated that some differences had been observed. Donefer (1966) called for establishment of standardized in vivo methods in order that large differences noted in variability of intake in different studies might be lessened. The small sample in vivo technique seems to overcome many of the difficulties encountered in the in vitro procedure. In addition, many small samples can be studied simultaneously. But as Pettyjohn (1964) pointed out the method needs testing with a large number of samples of different forages of known digestibilities. This would help to establish its precision and accuracy. The conventional digestion trial, although expensive and time consuming, will remain important as a direct method in forage studies. This is mostly because forage quality results obtained from indirect methods have to be compared with the results obtained in vivo on the same forage.

The literature contains conflicting results concerning the effects of the diet fed to the donor animal on the in vitro results. Until more information is available to clearly define the situation, it would seem to be better to feed the inoculum donor animal the same forage as that which will be analyzed in vitro. The studies reviewed indicate that fermentation time is an important factor in the in vitro technique. There is need, as Troelsen and Hanel (1966) observed, for the extension of the in vitro fermentation procedure to include an estimation of the fermentation time required to attain a digestibility similar to that obtainable in vivo. This time would be expected to vary depending on the type of forage and its stage of maturity.

The value and limitations of using regression equations to estimate in vivo digestibility from in vitro results were discussed. The literature reviewed indicates that legumes behave in a peculiar way when their in vitro results are related to in vivo values. The correlations are very low and in some cases are not significant. This then poses a problem when one wants to estimate in vivo values from in vitro results where legume-grass mixtures or legumes alone are involved.

From the literature, there is sufficient evidence to show that many investigators are not satisfied with the Weende proximate analysis scheme. In particular, the crude fiber and nitrogen-free-extract fractions of the scheme have been severely criticized as nutritionally unrealistic and not very meaningful. In spite of some of the limitations pointed out crude protein content is regarded as a useful indicator of forage value. Furthermore, compared to other components e.g. lignin, crude protein can be determined more quickly and easily on a routine basis.

There are indications in the literature that in trying to avoid using crude fiber as a measure of forage value, analysis for lignin, cellulose and hemicellulose is receiving increasing attention. One of the major problems in lignin determination is the efficient separation of protein from lignin without dissolving the latter. Information on the various fractions of the new method of feed analysis which is being investigated is presented in Table 1.

Evaluating forages on the basis of live weight gain or milk production may provide some useful indications on the value of a particular forage. But, as pointed out in this report, the investigator is

faced here with problems arising from the fact that two highly variable interacting biological systems are involved, namely the animal and the growing sward. The matter is further complicated if supplements are also fed and this is what happens under practical farm conditions. Reviews of work on palatability and animal preference, point to the need for further studies on the animal and plant factors involved.

The use of volatile fatty acids production as a measure of forage value is viewed with favor; and certainly in the case of ruminants the method has a sound theoretical basis since volatile fatty acids are a major digestion end product of forage. There are, however, practical problems caused by lack of sufficient understanding of the factors affecting the pattern of production of the volatile fatty acids with various feeds and at different levels of feeding.

Considering the literature reviewed in this report as a whole, there are no indications of a tendency among investigators to say that this or that is the method of forage evaluation. There is recognition of the ideal method and that is the response of the ruminant to the forage fed; but there is also recognition of the practical limitations involved in that method. Various research workers, therefore, are studying the different methods discussed in this report; noting the limitations of each method and its value to the central problem of forage evaluation.

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METHODS OF FORAGE EVALUATION

by

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AN ABSTRACT OF A MASTER'S REPORT

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The aim of this report was to summarize some of the results of the work which has been carried out concerning the various methods of forage evaluation.

From the literature on the in vivo digestibility studies there appeared to be a need for more studies to determine whether or not sheep and cattle have the same digestive ability. The use of fistulated animals in forage studies was examined and esophageally fistulated animals have been found to be particularly useful in sampling forages consumed by the grazing animal. The small sample in vivo technique was found to be a useful method in evaluation of small sample forages utilizing the natural environment of the rumen.

In the use of external indicators in digestibility studies, chromic oxide has been studied most. Its main limitations were, its low degree of recovery and a variable pattern of excretion.

Concerning the in vitro technique, conflicting results have been presented with regard to the effects of diet fed to the donor animal on the in vitro results. Until more information is available, it may be safer to feed the inoculum donor animal the same forage as that which will be analyzed in vitro. The opinion was expressed in the literature that in vitro fermentation studies should include an estimate of the fermentation time required to attain a digestibility similar to that obtainable in vivo. The use of in vitro results to estimate in vivo values was discussed.

There was evidence in the literature of a general dissatisfaction among investigators with the Weende proximate analysis scheme and in particular with the crude fiber and nitrogen-free-extract fractions

of the scheme. A proposed scheme of forage analysis was presented. The use of chemical composition results to estimate forage values was reviewed.

It was found that the use of milk production or liveweight gain data to estimate forage quality was limited by an incomplete understanding of the animal and plant factors which affect palatability and animal preference. The use of volatile fatty acids production in forage evaluation is favored by research workers. The use of this method, however, was limited by insufficient information on the factors affecting the pattern of production of the volatile fatty acids with various feeds and at different levels of feeding.